PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07K 7/10	A1	(11) International Publication Number: WO 89/09785 (43) International Publication Date: 19 October 1989 (19.10.89)

(21) International Application Number: PCT/US89/01426

(22) International Filing Date: 6 April 1989 (06.04.89)

(30) Priority data:
178,672 7 April 1988 (07.04.88) US
328,273 24 March 1989 (24.03.89) US

(71) Applicant: THE TRUSTEES OF THE UNIVERSITY OF ALABAMA [US/US]; University Station, Birmingham, AL 35294 (US).

(72) Inventors: COMPANS, Richard, W. ; Route 1, Box 406, Helena, AL 35080 (US). OWENS, Randy ; Route 2, Box 1407, Alabaster, AL 35007 (US).

(74) Agent: MISROCK, S., Leslie; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PEPTIDES INHIBITORS OF HIV INDUCED CELL FUSIONS AND SYNCYTIA FORMATION

(57) Abstract

Novel peptide sequences capable of inhibiting HIV-induced cell fusion are described. These peptides mimic regions within a hydrophobic domain located at the amino terminus of the transmembrane protein of the HIV envelope. Such sequences include the amino terminus of gp41 of HIV-1 and the amino terminus of gp40 of HIV-2. The peptides are at least two amino acids in length and can be used for the inhibition of HIV-induced fusion and syncytia formation which is a major cytopathological effect of HIV-infection *in vivo*.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland				

PEPTIDES INHIBITORS OF HIV INDUCED CELL FUSIONS AND SYNCYTIA FORMATION

1. INTRODUCTION

The present invention is directed to novel peptides which inhibit HIV-induced fusion and syncytia formation and, thus, inhibit one of the major cytopathological effects of HIV. The peptides mimic the amino terminus of the transmembrane protein of HIV virus. The invention is demonstrated by way of examples in which the efficacy of such inhibitory peptides is measured by an *in vitro* assay system.

2. BACKGROUND OF THE INVENTION

2.1. VIRAL GLYCOPROTEINS AND CELL FUSION

The entry of enveloped viruses into mammalian cells is thought to occur by a process of membrane fusion mediated by viral glycoproteins. Paramyxoviruses and members of the "type D" retrovirus group have been shown to exhibit fusion at neutral pH (Scheid and Choppin, 1974, Virology 57:475-490, and 1977, Virology 80: 54-66; Chatterjee et al., 1981, J. Virology 38:770-776). The paramyxoviruses deliver their nucleocapsids into the cytoplasm of the infected cells by fusion of the viral membrane with the plasma membrane. By contrast, orthomyxoviruses, rhabdoviruses, alphaviruses, and certain retroviruses have been demonstrated to enter the cell via coated pits which are internalized and become part of the endosomal compartment (White and Helenius, 1980 Proc. Natl. Acad. Sci. 77:3273-3277; White et al., 1980, J. Cell Biol. 87:264-273, and 1981, J. Cell Biol. 89:674-679; Redmond et al., 1984, Virology 133:393-402; Maeda and Ohnishi, 1980, FABS Letters 122:283-287; Huang et al., 1981, Virology 110:243-247). Following acidification of the endocytic vesicles, the fusion activity of specific viral glycoproteins is activated and causes fusion between the viral membrane and that of the endosomal vesicle membrane, resulting in the release of the nucleocapsids into the

cytoplasm. Agents that elevate endosomal and lysosomal pH, such as chloroquine or ammonium chloride, block infection by influenza and other acid-activated enveloped viruses (Jensen and Lui, 1961, Proc. Exper. Biol. Med. 107:447-451). Naturally occurring mutants of influenza virus which fuse at a pH higher than wild type virus were demonstrated to be more resistant to ammonium chloride (Doms et al., 1986, J. Virology 57:603-613). A recent study has suggested that HIV is internalized by receptor mediated endocytosis based on the finding that in the presence of ammonium chloride, there was greater than 95% inhibition of viral infection (Maddon et al., 1986, Cell 47:333-348). By contrast, a study performed by a different group, suggests that HIV gains entry into CD4-positive T cells by fusion (Stein et al., 1987, Cell 49:659-668).

The mechanism of membrane fusion induced by viral glycoproteins has been most extensively studied with the hemagglutinin (HA) glycoprotein of influenza virus and the F protein of Sendai virus (a paramyxovirus). The fusion activity, but not the receptor-binding activity, of the influenza hemagglutinin requires activation by proteolytic cleavage (by a host-specific enzyme) of an inactive precursor (HA_o) into two subunits (Lazarowitz and Choppin, 1975, Virology 68L:444-454). A similar cleavage is required to activate the F protein of Sendai virus (F_o) yeilding two disulfide-linked subunits $F_{1,2}$ (Scheid and Choppin, 1974, Virology 57:475-490; Huang et al., 1981, Virology 110:243-247). Cleavage of HA_o or F_o exposes hydrophobic amino termini on the HA_2 and F_1 subunits, which are thought to interact with the lipid bilayer of the target cell membrane and mediate fusion (Garten et al., 1981 Virology 115:361-374; Richardson et al., 1980, Virology 105:205-222). Cleavage of the F_o protein results in a conformational change as determined by a change in the circular dichroism and by increased binding of detergents, indicating an

exposed hydrophobic domain (Hsu et al., 1981, J. Biol. Chem. 256:3357-3363). Studies of the three-dimensional structure of influenza HA have demonstrated that in the neutral pH state, the HA molecule forms a trimer with the hydrophobic "fusion peptide" tucked into the interface between the subunits of the trimer (Wilson et al., 1981, Nature 289:366-373). It has been suggested that upon acidification, the hydrophobic "fusion peptide" is exposed and inserts into into the target cell membrane to mediate the fusion event (Daniels et al., 1985, Cell 40:431-439). Recently, studies using in vitro site directed mutagenesis to alter the hydrophobic amino terminus region of HA_2 have confirmed the importance of this region in the fusion process. Conversion of two glycine residues within the "fusion peptide" to glutamic acid residues either abolished fusion or increased the pH threshold and decreased the efficiency of fusion (Gething et al., 1986, J. Cell Biol. 102:11-23). Similarly, introduction of a charged residue at the N-terminus of the Sendai F_1 glycoprotein was found to block fusion activity (Hsu et al., 1981, J. Biol. Chem. 256:3547-3553). Oligopeptides with amino acid sequences corresponding to the amino terminus of influenza HA_2 or the Sendai F_1 were reported to be potent inhibitors of infectivity of each virus (Richardson et al., 1980, Virology 105:205-222; Richardson and Choppin, 1983, Virology 131:518-532). In addition, the oligopeptides corresponding to the amino terminus of F_1 of paramyxoviruses were shown to inhibit cell fusion and hemolysis (Richardson et al., 1980, Virology 105:205-222).

However, a recent publication (Hull et al., 1987, Virology 159:368-372) adds credence to an alternative to the "fusion-peptide" hypothesis for viral induced fusion. Hull et al. describe a measles virus mutant that is resistant to fusion inhibitory oligopeptides. Sequencing of the F glycoprotein revealed three amino acid changes, none of

which were located within the hydrophobic NH_2-terminal "fusion-peptide" region. The findings reported by Hull et al. suggest that the positioning of the F_1 amino terminus within a functional $F_{1,2}$ conformation, and not within the cell membrane, is the mediator of the fusion process.

2.2. HIV-INDUCED SYNCYTIA FORMATION

Human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), produces an envelope glycoprotein on its surface that is presumably responsible for viral entry into target cells. This glycoprotein is synthesized as a precursor of 160 Kd and is subsequently processed into two proteins, gp120 and gp41. The gp120 portion has been shown to bind directly to the cellular CD4 receptor molecule, hence producing HIV's tropism for host cells displaying the CD4 surface protein; whereas gp41 serves to anchor the envelope glycoprotein complex in the viral membrane. A prominent feature in the cytopathology of HIV infection is the formation of multinucleated syncytia (Barre-Sinoussi et al., 1983, Science 220:868-871; Popovic et al., 1984, Science 224:497-500; Levy et al., 1984, Science 225:840-842). A monoclonal antibody to the CD4 antigen was shown to inhibit fusion, indicating that binding to the CD4 antigen is required for syncytia formation (Lifson et al., 1986, Science 232:1123-1127). Recently, it was demonstrated that the envelope glycoprotein gene from HIV could be expressed in vaccinia virus vectors (Hu et al., 1986, Nature 320:537-540; Chakrabarti et al., 1986, Nature 320:535-537). This type of recombinant produced syncytium formation and cytopathology that were indistinguishable from that seen in HIV-infected cultures (Lifson et al., 1986, supra), demonstrating that the HIV gp120/gp41 glycoprotein when presented in a foreign virus "background" is capable of inducing syncytium

formation in the absence of other HIV proteins. However, the mechanism for such fusion remains to be elucidated.

To date, there is no cure for AIDS. The mechanism by which HIV induces its pathological effects is not well understood. A proposed mechanism is that CD4-positive cells are directly killed by viral replication (Montagnier, et al., 1984, In "Human T Cell Leukemia/Lymphoma Virus", Gallo, Essex and Gross, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. pp. 363-379). Vaccine trials are currently underway in an attempt to control the spread of the virus among the population. However, efforts at controlling the course of disease within an infected patient have been directed mainly towards the use of antiviral agents such as AZT (3'-azido-2',3'-dideoxythymidine), which interfere with the viral polymerase activity and, therefore, viral replication.

3. SUMMARY OF THE INVENTION

Novel peptide sequences capable of inhibiting HIV-induced cell fusion are described. The peptides of the invention mimic regions within a hydrophobic domain located at the amino terminus of the transmembrane protein of the HIV envelope. Such peptides have amino acid sequences that are homologous to this region found in various strains of HIV including but not limited to the amino terminus of gp41 of HIV-1 and the amino terminus of gp40 of HIV-2. The peptides are at least two amino acids in length and can be used for the inhibition of HIV-induced fusion and syncytia formation which is a major cytopathological effect of HIV-infection *in vivo*.

The peptides of the invention are demonstrated herein by way of examples in which the oligopeptides AVG and AVGIGA inhibited HIV-induced syncytia formation as measured by an *in vitro* assay using recombinant virus expressing

glycoproteins of the BH10 strain of HIV, and host cells that constitutively express the CD4 target antigen.

3.1. DEFINITIONS

Peptide sequences defined herein are represented by the one-letter symbols for amino acid residues as follows:

A (alanine), R (arginine), N (asparagine), D (aspartic acid), C (cysteine), Q (glutamine), E (glutamic acid), G (glycine), H (histidine), I (isoleucine), L (leucine), K (lysine), M (methionine), F (phenylalanine), P (proline), S (serine), T (threonine), W (tryptophan), Y (tyrosine), V (valine).

The following terms, as used herein, will have the meanings indicated:

a.a.	=	amino acid
HIV	=	human immunodeficiency virus (all variants)
h.p.i	=	hours post infection
m.o.i.	=	multiplicity of infection
p.f.u.	=	plaque forming units.

4. DESCRIPTION OF THE FIGURES

Figure 1. Imunoprecipitation, using AIDS patient antisera, of envelope glycoproteins in recombinant (VVenv) and wild type (w.t.) vaccinia virus-infected ^{3}H-leucine labeled HeLa T4 cells at 3, 6, and 10 hours post infection.

Figure 2. SDS polyacrylamide gel of ^{35}S-methionine labeled HeLa T4 cells at 12 hours post infection: (a) wild-type (w.t.) immunoprecipitate with patient antisera; (b) VVenv immunoprecipitate with patient antisera; (c) w.t. cell lysate; (d) VVenv cell lysate.

Figure 3. HeLa T4 cells infected with 1 p.f.u/cell VVenv at 7 hours post infection: (a) no peptide; (b) 8mM peptide AVGIGA; (c) 8mM peptide AVGAIG; and (d) 8mM peptide AVG.

Figure 4. Quantitation of peptide inhibition. HeLa T4 cells were infected at an m.o.i. of 0.1 with VVenv in the presence of various concentrations of each peptide and quantitated for syncytia at 20 hours post infection.

Figure 5. HeLa T4 cells infected with 1 p.f.u./cell, labeled with ^{35}S-methionine and immunoprecipitated using rabbit antisera at 10 hours post infection: (a) wild-type vaccinia; (b) VVenv, no peptide; (c) VVenv, 8mM peptide AVGIGA; (d) VVenv, 8mM peptide AVGAIG; (d) VVenv, 8mM peptide AVG.

5. DESCRIPTION OF THE INVENTION

Novel peptide sequences are described which are capable of inhibiting HIV-induced cell fusion and cytopathic syncytia formation. The invention is based, in part, on the discovery that peptides which are substantially homologous to the hydrophobic domain of the amino terminus of the transmembrane protein of HIV are effective inhibitors of HIV-induced fusion of infected cells.

5.1. PEPTIDE INHIBITORS OF HIV-INDUCED FUSION

The peptides of the invention have amino acid sequences that correspond to a region within a hydrophobic domain comprising approximately 21 to 40 amino acids located at the amino terminus of the transmembrane protein of the HIV envelope glycoprotein; for example, the first 21 to 30 amino acids of gp41 HIV-1, or the first 21-37 amino acids of gp40 of HIV-2. The complete genomic sequence of HIV-1 has been previously reported (Wain Hobson et al., 1985, Cell 40:9-17; Ratner et al., 1985, Nature 313:277-284; Sanchez-Pescador, et al., 1985, Science 227:484-492; and Starcich et al., 1986, Cell 45:637-648); as is the sequence for HIV-2 (Guyader et al., 1987, Nature 326:662-669). The peptides of the invention may comprise oligopeptides (e.g., at least two amino acid residues), polypeptides (e.g., more than 10 amino

acid residues) or proteins (e.g., polypeptides having a molecular weight of approximately 10 Kd or about 100 amino acid residues in length) which contain such sequences. Examples of the peptides of the invention are listed in Tables I through VI below, in which the amino acid sequences read from the amino terminus to the carboxyl terminus of each peptide.

TABLE I

PEPTIDES WHICH INHIBIT HIV-1-INDUCED FUSION

AMINO TERMINUS CARBOXY TERMINUS

```
Z_m-AV-X_n
Z_m-AVG-X_n
Z_m-AVG*-X_n
Z_m-AVG*I*-X_n
Z_m-AVG*I*G-X_n
Z_m-AVG*I*GA-X_n
Z_m-AVG*I*GAL-X_n
Z_m-AVG*I*GALF-X_n
Z_m-AVG*I*GALFL-X_n
Z_m-AVG*I*GALFLG-X_n
Z_m-AVG*I*GALFLGF-X_n
Z_m-AVG*I*GALFLGFL-X_n
Z_m-AVG*I*GALFLGFLG-X_n
Z_m-AVG*I*GALFLGFLGA-X_n
Z_m-AVG*I*GALFLGFLGAA-X_n
Z_m-AVG*I*GALFLGFLGAAG-X_n
Z_m-AVG*I*GALFLGFLGAAGS-X_n
Z_m-AVG*I*GALFLGFLGAAGST-X_n
Z_m-AVG*I*GALFLGFLGAAGSTM-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMG-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGA-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGAR-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARS-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSM-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMT-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTL-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLT-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTV-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTVQ-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
     I ALV  M              V L
        T                  A I
       IV                  G
```

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group (e.g., carboxybenzoxyl, dansyl or t-butyloxycarbonyl), or a cross reactive group (e.g. alkylating agent).

m and n each comprises an integer of at least 0.

Amino acids in boldface type may be inserted between amino acid residues at positions indicated above by * (when present) or substituted for the amino acid residue indicated directly above within any of the peptide sequences shown.

TABLE II

PEPTIDES WHICH INHIBIT HIV-1-INDUCED FUSION

AMINO TERMINUS → CARBOXY TERMINUS

Z_m-QA-X_n
Z_m-VQA-X_n
Z_m-TVQA-X_n
Z_m-LTVQA-X_n
Z_m-TLTVQA-X_n
Z_m-MTLTVQA-X_n
Z_m-SMTLTVQA-X_n
Z_m-RSMTLTVQA-X_n
Z_m-ARSMTLTVQA-X_n
Z_m-GARSMTLTVQA-X_n
Z_m-MGARSMTLTVQA-X_n
Z_m-TMGARSMTLTVQA-X_n
Z_m-STMGARSMTLTVQA-X_n
Z_m-GSTMGARSMTLTVQA-X_n
Z_m-AGSTMGARSMTLTVQA-X_n
Z_m-AAGSTMGARSMTLTVQA-X_n
Z_m-GAAGSTMGARSMTLTVQA-X_n
Z_m-LGAAGSTMGARSMTLTVQA-X_n
Z_m-FLGAAGSTMGARSMTLTVQA-X_n
Z_m-GFLGAAGSTMGARSMTLTVQA-X_n
Z_m-LGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-FLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-LFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-ALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-G*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-VG*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n

```
       I ALV  M                  V L
         T                       A I
         IV                      G
```

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group (*e.g.*, carboxybenzoxyl, dansyl or t-butyloxycarbonyl), or a cross reactive group (*e.g.* alkylating agent).

m and n each comprises an integer of at least 0.

Amino acids in boldface type may be inserted between amino acid residues at positions indicated above by * (when present) or substituted for the amino acid residue indicated directly above within any of the peptide sequences shown.

TABLE III

PEPTIDES WHICH INHIBIT HIV-2-INDUCED FUSION

AMINO TERMINUS	CARBOXY TERMINUS
Z_m-YS-X_n	
Z_m-YSS-X_n	
Z_m-YSSA-X_n	
Z_m-YSSAH-X_n	
Z_m-YSSAHG-X_n	
Z_m-YSSAHGR-X_n	
Z_m-YSSAHGRH-X_n	
Z_m-YSSAHGRHT-X_n	
Z_m-YSSAHGRHTR-X_n	
Z_m-YSSAHGRHTRG-X_n	
Z_m-YSSAHGRHTRGV-X_n	
Z_m-YSSAHGRHTRGVF-X_n	
Z_m-YSSAHGRHTRGVFV-X_n	
Z_m-YSSAHGRHTRGVFVL-X_n	
Z_m-YSSAHGRHTRGVFVLG-X_n	
Z_m-YSSAHGRHTRGVFVLGF-X_n	
Z_m-YSSAHGRHTRGVFVLGFL-X_n	
Z_m-YSSAHGRHTRGVFVLGFLG-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGF-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFL-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLA-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLAT-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATA-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAG-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGS-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSA-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAM-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMG-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGA-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAA-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAAS-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASL-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLT-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTV-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVS-X_n	
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n	

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group (e.g., carboxybenzoxyl, dansyl or t-butyloxycarbonyl), or a cross reactive group (e.g. alkylating agent).

m and n each comprises an integer of at least 0.

TABLE IV

PEPTIDES WHICH INHIBIT HIV-2-INDUCED FUSION

AMINO TERMINUS	CARBOXY TERMINUS
	Z_m-SA-X_n
	Z_m-VSA-X_n
	Z_m-TVSA-X_n
	Z_m-LTVSA-X_n
	Z_m-SLTVSA-X_n
	Z_m-ASLTVSA-X_n
	Z_m-AASLTVSA-X_n
	Z_m-GAASLTVSA-X_n
	Z_m-MGAASLTVSA-X_n
	Z_m-AMGAASLTVSA-X_n
	Z_m-SAMGAASLTVSA-X_n
	Z_m-GSAMGAASLTVSA-X_n
	Z_m-AGSAMGAASLTVSA-X_n
	Z_m-TAGSAMGAASLTVSA-X_n
	Z_m-ATAGSAMGAASLTVSA-X_n
	Z_m-LATAGSAMGAASLTVSA-X_n
	Z_m-FLATAGSAMGAASLTVSA-X_n
	Z_m-GFLATAGSAMGAASLTVSA-X_n
	Z_m-LGFLATAGSAMGAASLTVSA-X_n
	Z_m-FLGFLATAGSAMGAASLTVSA-X_n
	Z_m-GFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-LGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-VLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-FVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-VFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-GVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-RGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-TRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-HTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-RHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-GRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-HGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-AHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-SAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-SSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
	Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group (e.g., carboxybenzoxyl, dansyl or t-butyloxycarbonyl), or a cross reactive group (e.g. alkylating agent).

m and n each comprises an integer of at least 0.

TABLE V

PEPTIDES WHICH INHIBIT HIV-INDUCED FUSION

AMINO TERMINUS		CARBOXY TERMINUS
	Z_m-FL-X_n	
	Z_m-FLG-X_n	
	Z_m-FLGF-X_n	
	Z_m-FLGFL-X_n	
	Z_m-FLGFLG-X_n	
	Z_m-FLGFLGA-X_n	
	Z_m-FLGFLGAA-X_n	
	Z_m-FLGFLGAAG-X_n	
	Z_m-FLGFLGAAGS-X_n	
	Z_m-FLGFLGAAGST-X_n	
	Z_m-FLGFLGAAGSTM-X_n	
	Z_m-FLGFLGAAGSTMG-X_n	
	Z_m-FLGFLGAAGSTMGA-X_n	
	AT **AV**	

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group (e.g., carboxybenzoxyl, dansyl or t-butyloxycarbonyl), or a cross reactive group (e.g. alkylating agent).

m and n each comprises an integer of at least 0.

Amino acids in boldface type may be inserted between amino acid residues at positions indicated above by * (when present) or substituted for the amino acid residue indicated directly above within any of the peptide sequences shown.

TABLE VI

PEPTIDES WHICH INHIBIT HIV-INDUCED FUSION

AMINO TERMINUS		CARBOXY TERMINUS
	Z_m-GA-X_n	
	Z_m-MGA-X_n	
	Z_m-TMGA-X_n	
	Z_m-STMGA-X_n	
	Z_m-GSTMGA-X_n	
	Z_m-AGSTMGA-X_n	
	Z_m-AAGSTMGA-X_n	
	Z_m-GAAGSTMGA-X_n	
	Z_m-LGAAGSTMGA-X_n	
	Z_m-FLGAAGSTMGA-X_n	
	Z_m-GFLGAAGSTMGA-X_n	
	Z_m-LGFLGAAGSTMGA-X_n	
	Z_m-FLGFLGAAGSTMGA-X_n	
	AT **AV**	

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group (e.g., carboxybenzoxyl, dansyl or t-butyloxycarbonyl), or a cross reactive group (e.g. alkylating agent).

m and n each comprises an integer of at least 0.

Amino acids in boldface type may be inserted between amino acid residues at positions indicated above by * (when present) or substituted for the amino acid residue indicated directly above within any of the peptide sequences shown.

Although the peptides of the invention are synthesized to resemble the amino-terminal region of the HIV transmembrane protein, variations in the amino acid sequence, the steric configuration, the type of covalent bond which links the amino acid residues, and/or addition of groups to the amino- or carboxy-terminal residues are within the scope of the invention. For example, the peptides of the invention may include altered sequences to accomodate strain-to-strain variations among different HIV isolates as well as conservative alterations which result in a silent change thus producing a functionally equivalent peptide. Thus, the peptides depicted in Tables I through VI may be altered by various changes such as insertions, deletions and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use. As used herein, conservative substitutions would involve the substitution of one or more amino acids within the peptide sequences shown in Tables I through VI with another amino acid having similar polarity and hydrophobicity/hydrophilicity characteristics resulting in a silent alteration and a functionally equivalent molecule. Such conservative substitutions incude but are not limited to substitutions within the following groups of amino acids: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; phenylalanine, tyrosine; and methionine, norleucine.

The steric configuration of the peptides may be altered in order to increase the inhibitory activity of the peptides. For example, the use of a D-isomer rather than the L-isomer at the amino terminus of the peptide and/or opposite configurations of the first two amino acids of the peptide may provide for an increased inhibitory function. Such variations in the steric configuration may enhance inhibitory activity by protecting the peptide from

proteolysis or by affecting the affinity of the peptide for its binding site.

Additional amino acids or other chemical groups may be added to either the amino or carboxy terminus of the peptides in order to alter or enhance their activity. For example, the addition of hydrophobic groups such as carbobenzoxyl, dansyl or t-butyloxycarbonyl to the amino terminus may increase the inhibitory activity of the peptides. Similarly, the addition of t-butyloxycarbonyl to the carboxy terminus may also increase inhibitory activity. By contrast, the addition of groups such as carbobenzoxyl or dansyl to the carboxy terminus may have less effect, or may result in a decrease in inhibitory activity.

Alternatively, peptides derivatized by the addition of chemically reactive groups that allow for the formation of covalent bonds between the peptides of the invention and their binding sites may be prepared. Such peptides would form covalent bonds with their binding sites and bind irreversibly to their target. For example, the peptides may be derivatized with an alkylating agent such as chloromethylketone (Powers, 1980, in Methods in Enzymology, Jakoby and Wilkek, eds, Acad. Press, N.Y., Vol. 46:197-208). Such derivatives are essentially alkylating agents that can react with and modify histidine, serine and sulfhydryl residues thus allowing the formation of covalent bonds between the inhibitory peptide and its binding site on the target cell.

The peptides of the invention may be synthesized or prepared by any technique known in the art. Short peptides can be synthesized on a solid support or in solution in accordance with conventional techniques. Commercially available automated synthesizers may be conveniently used. See, for example, Merrifield, 1969, Adv. Enzymol. Relat. Areas Mol. Bio. 32:221-296; Tam et al., 1983, J. Am. Chem.

Soc. 105:6442; Konig and Geiger, 1970, Chem. Ber. 103:788-789 and 2034-2040).

Alternatively, longer peptides may be produced using recombinant DNA technology. Accordingly, the nucleotide coding sequences for the peptides of the invention may be cloned and expressed using techniques well known in the art. See, for example, Maniatus et al., Molecular Cloning, A Laboratory Manual, CSH, Cold Spring Harbor Laboratory, 1982.

In another embodiment of the invention, peptide analogs may be synthesized which comprise the sequences shown and described _supra_ in which the amino acid residues are linked one to the other by non-peptide covalent bonds. Such covalent linkages can be made using reactions well known in the art involving the amino and/or carboxyl groups of the amino acids as well as any other reactive group, (_e.g._ sulfur) that may be present. The non-peptide (or non-amide) linkages which can be used to synthesize these peptide analogos of the invention include but are not limited to imino, ester, hydrazide, semicarbazide, and azo bonds to name but a few.

5.2. IN VITRO ASSAY FOR DEMONSTRATING EFFECT OF OLIGOPEPTIDE INHIBITORS OF HIV-INDUCED SYNCYTIA FORMATION

The inhibitory activity of the peptides can be measured and the functional equivalency and/or increased efficacy of the altered peptides can readily be tested using the _in vitro_ assay system described in Section 6.2 herein. Accordingly, the efficacy of any peptide of the invention may be assessed by its relative ability to inhibit the formation of syncytia _in vitro_ using a cell line that constitutively expresses the CD4 receptor molecule and a recombinant vaccinia virus that expresses the HIV _env_ glycoprotein. These recombinant viruses induce the formation of syncytia which are morphologically

indistinguishable from those formed by authentic HIV. The ability of the peptides or altered peptides of the invention to inhibit syncytia formation in this assay system is indicative of the inhibitory activity of these peptides against authentic HIV-induced fusion.

5.2.1. RECOMBINANT VACCINIA VIRUS

The assay system described herein which utilizes a recombinant vaccinia virus that expresses the HIV glycoprotein offers several advantages for characterizing the peptides of the invention and for screening antiviral agents which may be useful against HIV-infection. In particular, the efficacy of inhibitory and/or antiviral compounds can be assessed without risking exposure to authentic HIV and thus eliminates the possibility of contracting AIDS.

Moreover the recombinant vaccinia virus described in the Example of Section 6.2 was constructed using the IHD strain of vaccinia. This vaccinia strain, unlike the WR strain which has commonly been used to construct recombinants demonstrates increased levels of expression of the foreign gene inserted into the virus, and has a decreased cytopathic effect. As a result, the recombinant vaccinia can be used to infect the target test cells and results in a greatly increased level of surface expression of the HIV envelope glycoprotein at shorter times after infection. In addition, synchronous expression in culture is achieved. Finally, the decreased cytopathic effect of the IHD vaccinia strain may reduce the risks for the technicians who must handle the recombinants and run the assay system making the assay system described herein very desirable for evaluation, assessment characterization and screening of inhibitory and/or antiviral compounds. The properties of this vaccinia virus also makes the recombinant

virus exemplified herein a desirable candidate for potential use in vaccines.

5.3. USES OF INHIBITORY PEPTIDES OF THE INVENTION

The inhibitory peptides of the invention may be used *in vivo* to prevent the formation of syncytia and, thus, inhibit the progression of HIV infection within an exposed patient. Effective doses of the peptides of the invention formulated in suitable pharmacological carriers may be administered by any appropriate route including but not limited to injection (*e.g.*, intravenous, intraperitoneal, intramuscular, subcutaneous, etc.), by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and vaginal epithelial linings, nasopharyngeal mucosa, intestinal mucosa, etc); etc.

The peptides may be mixed in any suitable pharmacological carrier, linked to a carrier molecule and/or incorporated into liposomes, microcapsules, and controlled release preparations prior to administration *in vivo*.

The peptides of the invention may also be used to identify different strains of HIV. For example, the peptides of the invention will exert an inhibitory effect on HIV strains that have transmembrane proteins which share the sequence specificity of the peptide. By contrast, an HIV strain with a very divergent transmembrane sequence may not be inhibited. Therefore, the peptides having known sequences could be used in an *in vitro* assay to quickly determine whether a particular strain of HIV isolated from a patient is a new variant. Accordingly, a cell line which constitutively expresses the CD4 receptor, such as the HeLa T4 cell line of the Examples described in Section 6.2 *infra*, would be used as the target cells *in vitro*. HIV isolated from a patient can be used to infect the CD4 positive cell line *in vitro* in the presence of a peptide of the invention which is specific for a particular strain of HIV. Failure

of the peptide to inhibit virus-induced fusion would indicate that the HIV isolate is a different strain.

6. EXAMPLE: SELECTIVE INHIBITION OF HIV-1 ENVELOPE GLYCOPROTEIN-INDUCED SYNCYTIUM FORMATION

The human immunodeficiency virus (HIV-1) envelope glycoprotein is essential for virus entry and the formation of multinucleated giant cells by cell fusion, the major virus-induced cytopathic effect. The subsections below describe (a) oligopeptides which inhibit HIV-induced fusion; and (b) an *in vitro* assay system devised to determine the effects of potential fusion inhibitors. For the *in vitro* assay system, a recombinant vaccinia virus expressing the envelope glycoprotein of the BH-10 strain of HIV was generated and used to infect a HeLa cell line (HeLa T4) which constitutively expresses the CD4 receptor molecule. Syncytium induction in this system was observed as early as 4 hours post-infection and continued until the entire monolayer was fused. We observed that the N-terminus of the gp41 subunit of the HIV envelope protein is very hydrophobic, and we considered it likely that it may be involved in virus-induced cell fusion. We synthesized several oligopeptides homologous to the N-terminal region of gp41 and determined their ability to inhibit HIV-1-induced cell fusion in the HeLa T4 cell system. A hexapeptide which was identical in amino acid sequence to the N-terminus of gp41 of the BH-10 isolate was found to almost completely prevent virus-induced cell fusion. In contrast, a peptide which differed by a single amino acid insertion showed almost no inhibitory effect against the BH-10 isolate. These results indicate that oligopeptides which are homologous to the fusion peptide of HIV inhibit virus-induced cytopathology.

6.1. OLIGOPEPTIDE INHIBITORS

Three synthetic peptides were made with varying degrees of homology to the N-terminal amino acids of gp41 from different HIV strains. The first peptide:

$$Ala_1\text{-}Val_2\text{-}Gly_3\text{-}Ile_4\text{-}Gly_5\text{-}Ala_6 \text{ (AVGIGA)}$$

was identical in sequence to the first six amino acids of gp41 of the BH10 strain.

The second peptide:

$$Ala_1\text{-}Val_2\text{-}Gly_3\text{-}Ala_4\text{-}Ile_5\text{-}Gly_6 \text{ (AVGAIG)}$$

varied slightly in the order of amino acids 4, 5 and 6 and was homologous to the N-terminus of the WMJ-1 varient of gp41.

Because amino acids 1, 2, and 3 of the first two peptides were identical, a tripeptide with the following sequence was synthesized:

$$Ala_1\text{-}Val_2\text{-}Gly_3 \text{ (AVG)}$$

6.1.1. SYNTHESIS OF OLIGOPEPTIDES

Peptides were synthesized by the solid phase procedure using an Applied Biosystems model 430A instrument. The completed peptides were removed from the resin and purified by high performance liquid chromatography.

6.2. IN VITRO ASSAY FOR VIRUS-INDUCED SYNCYTIA FORMATION

The effects of each oligopeptide synthesized in Section 6.1 on HIV-induced cell fusion was assayed as described in the subsections below. In general, cell fusion was assayed using a HeLa cell line (HeLa T4) which constitutively expresses the CD4 receptor molecule. The recombinant vaccinia virus (VV-env-1) which expresses the

HIV (BH10 strain) envelope glycoprotein induces the formation of syncytia in the HeLa T4 cell line.

6.2.1. RECOMBINANT VACCINIA VIRUS

The general procedures used to construct the recombinant vaccinia viruses used in the Examples *infra* are summarized as follows (for review see Smith and Moss, 1983, Gene 25:21-28): To facilitate the construction of vaccinia recombinants, several plasmid vectors termed insertion vectors were used to insert the foreign gene into vaccinia virus via homologous recombination *in vivo* (Mackett et al., 1984, J. Virol. 49:857-864; Smith et al., 1983, Nature 302:490-495 and Proc. Natl. Acad. Sci. 80:7155-7159). In addition to possessing a bacterial origin of DNA replication and antibiotic resistance marker, these insertion plasmids contain an isolated vaccinia virus promoter including the transcriptional initiation site and 200-300 base pairs of upstream DNA, with several unique restriction endonuclease cleavage sites positioned downstream from the transcriptional start point; both sides of the promoter and restriction sites are flanked by nonessential vaccinia virus DNA that directs insertion of the foreign DNA into a homologous nonessential region of the viral genome. After construction of a plasmid containing HIV DNA by cleavage and ligation, virus recombinants were generated by transfection of these plasmids into cells infected with vaccinia virus. By insertion of such foreign DNA within the vaccinia virus TK gene, TK^- mutants are obtained which can be identified by their ability to be grown on TK^- host cells in the presence of BUdR. Recombinants may be further distinguished from wild type parental virus by screening of plaques with a labeled DNA probe specific for the foreign gene, immunological analysis using antiserum specific for the foreign protein and ^{125}I-labeled protein A from *S. aureus* or a second antibody, or color screening by use of an insertion

vector containing the β-galactosidase gene of E. coli. After incubation with the chromogenic substrate 5-bromo-4-chloro-3-indolyl-B-D-galactopyranoside (X-gal), recombinant plaques expressing β-galactosidase activity become blue. After at least two cycles of plaque purification, recombinant virus stocks were prepared in any of a variety of possible host cell types. A plasmid insertion vector was used to construct the recombinant vaccinia viruses described in more detail below.

The envelope gene was obtained from plasmid pBH10 and inserted into the vaccinia recombinant plasmid vector pSC11. The plasmid pBH-10 (Ratner et al., 1985, Nature 313:277-284) contains a DNA clone of the unintegrated HTLV-III genome in the Sst I site of plasmid pSP65. The entire envelope gene was subcloned by digesting pBH10 with SalI and XhoI followed by gel isolation of the 3.1 kilobase pair fragment. This fragment was ligated to the SalI digested plasmid vector pGem-3 (Promega Biotech, Madison, WI). Orientation of the env gene was determined by restriction analysis. After cleavage with SacI and SalI, unindirectional digestion with exonuclease III and S1 nuclease was carried out. Plasmids were recircularized with T4 ligase and used to transform E. coli HB101 cells. Plasmids from individual colonies were sequenced by the dideoxy method to determine that 56 nucleotides remained upstream of the translation initiation site of the env gene. DNA fragments containing the entire env gene were obtained by cleavage with EcoRI and PstI, after which blunt ends were created using the Klenow fragment of DNA polymerase and deoxyribonucleoside triphosphate. These fragments were inserted into the SmaI site of pSC11 containing the β-galactosidase gene (Chakrabarti et al., 1985, J. Virol. 5:3043-3049). After transfection into HB101 cells, and screening with an env gene probe by colony blot hybridization and restriction enzyme analysis, a plasmid designated pVenv-1 was obtained.

For insertion into vaccinia virus, TK^-143 cells were infected with the IHD strain of vaccinia at an m.o.i. of 0.1 p.f.u./cell. At 2 h.p.i., cells were transfected with a calcium phosphate precipitate of 5 μg pVenv-1 and 15 μg of salmon sperm DNA/ml of Hepes-buffered saline. Stocks of TK^- vaccinia virus were prepared in TK^-143 cells (Smith and Moss, 1983, Gene 25:21-28). To select for recombinants, TK^-143 cells were infected with 100-200 p.f.u. of TK^- vaccinia virus in the presence of BUdR (25 μg/ml). At 48 hr after infection, the monolayers were overlayed with 1% low melting agarose containing 300 μg/ml 5-bromo-4-chloro-3-indolyl-B-D-galactopyranoside (Xgal). At 4-6 hours, blue plaques were picked and further purified by two additional rounds of plaque purification. Plaque-purified virus was grown in CV-1 cells and purified as described by Joklik (1962, Biochem. Biophys. Acta 61:290-301). Recombinant vaccinia virus was generated as previously described (Stephens and Compans, 1986, Cell 47:1053-1059).

Synthesis of the HIV-1 env protein was demonstrated by immunoprecipitation of lysates of cells infected with the recombinant vaccinia virus (designated VVenv-1) using AIDS patient antisera followed by polyacrylamide gel electrophoresis (FIG. 1). A strong band which corresponds to the precursor gp160, and two weaker bands migrating at about 120 Kd and 40 Kd were observed in the recombinant infected cells. The gp120 molecule appears as a major band in total cell lysates indicating that proteolytic processing is occurring and that the recombinant vaccinia virus expresses the envelope glycoprotein very efficiently (FIG. 2). The antisera used appeared to be more reactive with gp41, which would explain the low reactivity with gp120 and high reactivity with gp160.

6.2.2. INFECTION OF CELL LINE THAT CONSTITUTIVELY EXPRESS THE CD4 RECEPTOR

We obtained a HeLa cell line (HeLa T4) that constitutively expresses the CD4 receptor molecule to test whether or not the recombinant vaccinia virus was capable of inducing cell fusion (Maddon et al., 1986, Cell 47:333-348). When infected at an m.o.i. of 5-10, focal areas of fusion containing 2-4 nuclei were observed as early as 4 h.p.i. and by 12 h.p.i. the entire monolayer of cells was fused together. Indirect surface immunofluorescence showed the presence of the envelope glycoprotein on the surface of multinucleated giant cells (FIG. 3).

6.3. INHIBITORY EFFECT OF OLIGOPEPTIDES OF THE INVENTION

The effects of each oligopeptide synthesized in Section 6.1 on HIV replication and virus-induced cell fusion were assayed using the system described in Section 6.2. A range of concentrations (0.5 mM to 8mM) were analyzed for each peptide tested. The appropriate concentration of peptide was added to the HeLa T4 cells one hour post infection with VV*env*-1. The results of this analysis, described in detail below, indicate that the peptides AVGIGA and AVG were effective in inhibiting syncytium formation induced by HIV. Immunoprecipitation and immunofluorescence analysis indicated that the inhibitory effect of these peptides did not reside at the level of protein synthesis, proteolytic processing or protein transport.

6.3.1. OLIGOPEPTIDES INHIBIT HIV-INDUCED SYNCYTIUM FORMATION

The peptides synthesized in Section 6.1 were tested for their ability to inhibit syncytium formation in HeLa T4 cells infected with a recombinant vaccinia virus that expresses the glycoprotein of the BH10 strain of HIV. The greatest degree of inhibition was observed with peptide

AVGIGA (derived from the transmembrane protein of the BH10 strain of HIV), while peptide AVGAIG (derived from the transmembrane protein of the WMJ strain of HIV) showed a lesser inhibitory effect. Peptide AVG exhibited an inhibitory effect that was between that observed for peptide AVGIGA and peptide AVGAIG (FIG. 4). The I_{50} (concentration at which 50% syncytium are inhibited) for peptides AVGIGA and AVG were both about 0.75 mM and the I_{50} for peptide AVGAIG was about 2.5 mM. At 0.75 mM, peptide AVGIGA was about 2.5-fold more effective than peptide AVGAIG at inhibiting cell fusion. At the highest concentration tested (8 mM) peptide AVGIGA was slightly more effective than both peptide and AVGAIG peptide AVG.

6.3.2. OLIGOPEPTIDES DO NOT INHIBIT PROTEIN SYNTHESIS AND PROCESSING OF GP160

To determine whether these peptides were exerting an inhibitory effect on protein synthesis and processing, VVenv1 infected HeLa T4 cells were radiolabeled with ^{3}H-leucine in the presence of inhibitory concentration of each peptide, and immunoprecipitated for analysis of env glycoprotein synthesis by polyacrylamide gel electrophoresis (FIG. 5). No differences were observed in the level of env gene products in the presence or absence of peptide indicating that the inhibitory effects of these peptides do not reside at the level of protein synthesis or proteolytic processing of gp160.

6.3.3. OLIGOPEPTIDES DO NOT BLOCK TRANSPORT OF GLYCOPROTEIN TO CELL SURFACE

To determine whether the peptides were inhibiting transport of the env glycoprotein to the cell surface, HeLa T4 cells were infected with VVenv1 and analyzed for surface expression by indirect surface immunofluorescence (FIG. 3). The relative fluorescence intensities of cells treated with

high concentrations of each peptide were the same as cells that received no peptide, indicating that syncytium inhibition was not due to a block in transport of the glycoprotein to the cell surface.

7. DEPOSIT OF MICROORGANISMS

The following and recombinant viruses have been deposited with the American Type Culture Collection, Rockville, MD, and have been assigned the listed accession numbers:

Recombinant Virus	Accession No.
VV-env-1	ATCC 2208

The present invention is not to be limited in scope by the cell lines and viruses deposited and peptides exemplified which are intended as but single illustrations of one aspect of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 29, line 6-23 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

American Type Culture Collection

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, MD 20852

Date of deposit [5]	Accession Number [6]
April 5, 1988	VR 2208

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [8] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☒ This sheet was received with the international application when filed (to be checked by the receiving Office)

Nathaniel R. Hayden
PCT INTERNATIONAL SERVICES DIVISION
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

WHAT IS CLAIMED IS:

1. A peptide comprising the formula selected from the group consisting of:

Z_m-AV-X_n
Z_m-AVG-X_n
Z_m-AVG*-X_n
Z_m-AVG*I*-X_n
Z_m-AVG*I*G-X_n
Z_m-AVG*I*GA-X_n
Z_m-AVG*I*GAL-X_n
Z_m-AVG*I*GALF-X_n
Z_m-AVG*I*GALFL-X_n
Z_m-AVG*I*GALFLG-X_n
Z_m-AVG*I*GALFLGF-X_n
Z_m-AVG*I*GALFLGFL-X_n
Z_m-AVG*I*GALFLGFLG-X_n
Z_m-AVG*I*GALFLGFLGA-X_n
Z_m-AVG*I*GALFLGFLGAA-X_n
Z_m-AVG*I*GALFLGFLGAAG-X_n
Z_m-AVG*I*GALFLGFLGAAGS-X_n
Z_m-AVG*I*GALFLGFLGAAGST-X_n
Z_m-AVG*I*GALFLGFLGAAGSTM-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMG-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGA-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGAR-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARS-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSM-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMT-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTL-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLT-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTV-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTVQ-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n

I ALV M V L
T A I
IV G

in which:

amino acid residues are presented by the single-letter code;

Z and X, when present, each comprises one or more amino acid residue, a hydrophobic group, or a cross reactive group;

m and n each comprises an integer of at least 0; and

bold face letters comprise amino acids which may be inserted between amino acid residues at positions

indicated above by *, when present, or substituted for the residue indicated above, within each peptide sequence.

2. The peptide according to claim 1 in which Z comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

3. The peptide according to claim 1 in which Z comprises an alkylating agent.

4. The peptide according to claim 3 in which the alkylating agent comprises chloromethylketone.

5. The peptide according to claim 1 in which X comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

6. The peptide according to claim 1 in which X comprises an alkylating agent.

7. The peptide according to claim 6 in which the alkylating agent comprises chloromethylketone.

8. A peptide comprising the formula selected from the group consisting of:

```
Z_m-QA-X_n
Z_m-VQA-X_n
Z_m-TVQA-X_n
Z_m-LTVQA-X_n
Z_m-TLTVQA-X_n
Z_m-MTLTVQA-X_n
Z_m-SMTLTVQA-X_n
Z_m-RSMTLTVQA-X_n
Z_m-ARSMTLTVQA-X_n
Z_m-GARSMTLTVQA-X_n
Z_m-MGARSMTLTVQA-X_n
Z_m-TMGARSMTLTVQA-X_n
Z_m-STMGARSMTLTVQA-X_n
Z_m-GSTMGARSMTLTVQA-X_n
Z_m-AGSTMGARSMTLTVQA-X_n
Z_m-AAGSTMGARSMTLTVQA-X_n
Z_m-GAAGSTMGARSMTLTVQA-X_n
Z_m-LGAAGSTMGARSMTLTVQA-X_n
Z_m-FLGAAGSTMGARSMTLTVQA-X_n
Z_m-GFLGAAGSTMGARSMTLTVQA-X_n
Z_m-LGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-FLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-LFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-ALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-G*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-VG*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
Z_m-AVG*I*GALFLGFLGAAGSTMGARSMTLTVQA-X_n
     I ALV  M              V L
       T                   A I
       IV                  G
```

in which:

amino acid residues are presented by the single-letter code;

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group, or a cross reactive group;

m and n each comprises an integer of at least 0; and

bold face letters comprise amino acids which may be inserted between amino acid residues at positions indicated above by *, when present, or

substituted for the residue indicated above, within each peptide sequence.

9. The peptide according to claim 8 in which Z comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

10. The peptide according to claim 8 in which Z comprises an alkylating agent.

11. The peptide according to claim 10 in which the alkylating agent comprises chloromethylketone.

12. The peptide according to claim 8 in which X comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

13. The peptide according to claim 8 in which X comprises an alkylating agent.

14. The peptide according to claim 13 in which the alkylating agent comprises chloromethylketone.

15. A peptide comprising the formula selected from the group consisting of:

Z_m-YS-X_n
Z_m-YSS-X_n
Z_m-YSSA-X_n
Z_m-YSSAH-X_n
Z_m-YSSAHG-X_n
Z_m-YSSAHGR-X_n
Z_m-YSSAHGRH-X_n
Z_m-YSSAHGRHT-X_n
Z_m-YSSAHGRHTR-X_n
Z_m-YSSAHGRHTRG-X_n
Z_m-YSSAHGRHTRGV-X_n
Z_m-YSSAHGRHTRGVF-X_n
Z_m-YSSAHGRHTRGVFV-X_n
Z_m-YSSAHGRHTRGVFVL-X_n
Z_m-YSSAHGRHTRGVFVLG-X_n
Z_m-YSSAHGRHTRGVFVLGF-X_n
Z_m-YSSAHGRHTRGVFVLGFL-X_n
Z_m-YSSAHGRHTRGVFVLGFLG-X_n
Z_m-YSSAHGRHTRGVFVLGFLGF-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFL-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLA-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLAT-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATA-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAG-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGS-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSA-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAM-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMG-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGA-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAA-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAAS-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASL-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLT-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTV-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVS-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n

in which:

amino acid residues are presented by the single-letter code;

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group, or a cross reactive group; and

m and n each comprises an integer of at least 0.

16. The peptide according to claim 15 in which Z comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

17. The peptide according to claim 15 in which Z comprises an alkylating agent.

18. The peptide according to claim 17 in which the alkylating agent comprises chloromethylketone.

19. The peptide according to claim 15 in which X comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

20. The peptide according to claim 15 in which X comprises an alkylating agent.

21. The peptide according to claim 20 in which the alkylating agent comprises chloromethylketone.

22. A peptide comprising the formula selected from the group consisting of:

Z_m-SA-X_n
Z_m-VSA-X_n
Z_m-TVSA-X_n
Z_m-LTVSA-X_n
Z_m-SLTVSA-X_n
Z_m-ASLTVSA-X_n
Z_m-AASLTVSA-X_n
Z_m-GAASLTVSA-X_n
Z_m-MGAASLTVSA-X_n
Z_m-AMGAASLTVSA-X_n
Z_m-SAMGAASLTVSA-X_n
Z_m-GSAMGAASLTVSA-X_n
Z_m-AGSAMGAASLTVSA-X_n
Z_m-TAGSAMGAASLTVSA-X_n
Z_m-ATAGSAMGAASLTVSA-X_n
Z_m-LATAGSAMGAASLTVSA-X_n
Z_m-FLATAGSAMGAASLTVSA-X_n
Z_m-GFLATAGSAMGAASLTVSA-X_n
Z_m-LGFLATAGSAMGAASLTVSA-X_n
Z_m-FLGFLATAGSAMGAASLTVSA-X_n
Z_m-GFLGFLATAGSAMGAASLTVSA-X_n
Z_m-LGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-VLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-FVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-VFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-GVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-RGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-TRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-HTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-RHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-GRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-HGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-AHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-SAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-SSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n
Z_m-YSSAHGRHTRGVFVLGFLGFLATAGSAMGAASLTVSA-X_n

in which:

amino acid residues are presented by the single-letter code;

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group, or a cross reactive group; and

m and n each comprises an integer of at least 0.

23. The peptide according to claim 22 in which Z comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

24. The peptide according to claim 22 in which Z comprises an alkylating agent.

25. The peptide according to claim 24 in which the alkylating agent comprises chloromethylketone.

26. The peptide according to claim 22 in which X comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

27. The peptide according to claim 22 in which X comprises an alkylating agent.

28. The peptide according to claim 27 in which the alkylating agent comprises chloromethylketone.

29. A peptide comprising the formula selected from the group consisting of:

Z_m-FL-X_n
Z_m-FLG-X_n
Z_m-FLGF-X_n
Z_m-FLGFL-X_n
Z_m-FLGFLG-X_n
Z_m-FLGFLGA-X_n
Z_m-FLGFLGAA-X_n
Z_m-FLGFLGAAG-X_n
Z_m-FLGFLGAAGS-X_n
Z_m-FLGFLGAAGST-X_n
Z_m-FLGFLGAAGSTM-X_n
Z_m-FLGFLGAAGSTMG-X_n
Z_m-FLGFLGAAGSTMGA-X_n
AT AV

in which:

amino acid residues are presented by the single-letter code;

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group, or a cross reactive group;

m and n each comprises an integer of at least 0; and

bold face letters comprise amino acids which may be inserted between amino acid residues at positions indicated above by *, when present, or substituted for the residue indicated above, within each peptide sequence.

30. The peptide according to claim 29 in which Z comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

31. The peptide according to claim 29 in which Z comprises an alkylating agent.

32. The peptide according to claim 31 in which the alkylating agent comprises chloromethylketone.

33. The peptide according to claim 29 in which X comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

34. The peptide according to claim 29 in which X comprises an alkylating agent.

35. The peptide according to claim 33 in which the alkylating agent comprises chloromethylketone.

36. A peptide comprising the formula selected from the group consisting of:

Z_m-GA-X_n
Z_m-MGA-X_n
Z_m-TMGA-X_n
Z_m-STMGA-X_n
Z_m-GSTMGA-X_n
Z_m-AGSTMGA-X_n
Z_m-AAGSTMGA-X_n
Z_m-GAAGSTMGA-X_n
Z_m-LGAAGSTMGA-X_n
Z_m-FLGAAGSTMGA-X_n
Z_m-GFLGAAGSTMGA-X_n
Z_m-LGFLGAAGSTMGA-X_n
Z_m-FLGFLGAAGSTMGA-X_n
AT **AV**

in which:

amino acid residues are presented by the single-letter code;

Z and X, when present, each comprises one or more amino acid residues, a hydrophobic group, or a cross reactive group;

m and n each comprises an integer of at least 0; and

bold face letters comprise amino acids which may be inserted between amino acid residues at positions indicated above by *, when present, or substituted for the residue indicated above, within each peptide sequence.

37. The peptide according to claim 36 in which Z comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

38. The peptide according to claim 36 in which Z comprises an alkylating agent.

39. The peptide according to claim 38 in which the alkylating agent comprises chloromethylketone.

40. The peptide according to claim 36 in which X comprises a hydrophobic group selected from the group consisting of carboxybenzoxyl, dansyl, and t-butyloxycarbonyl.

41. The peptide according to claim 36 in which X comprises an alkylating agent.

42. The peptide according to claim 41 in which the alkylating agent comprises chloromethylketone.

43. A peptide which is substantially homologous to a hydrophobic domain of the amino terminus of the transmembrane protein of the human immunodeficiency virus and which inhibits virus-induced fusion of infected cells.

44. The peptide of claim 43 which is substantially homologous to the amino terminus of gp41 of HIV-1.

45. The peptide of claim 43 which is substantially homologous to the amino terminus of gp40 of HIV-2.

FIG. 2

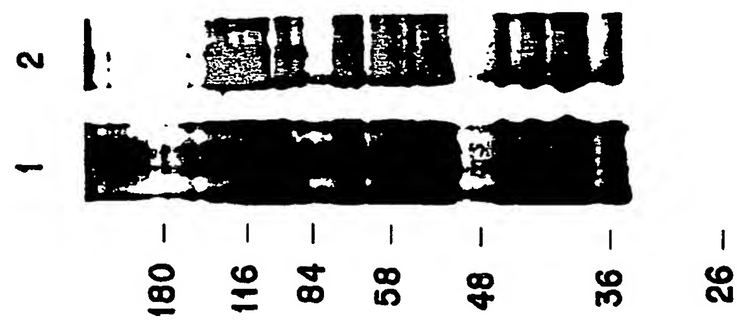

1
2
180
116
84
58
48
36
26

FIG. I

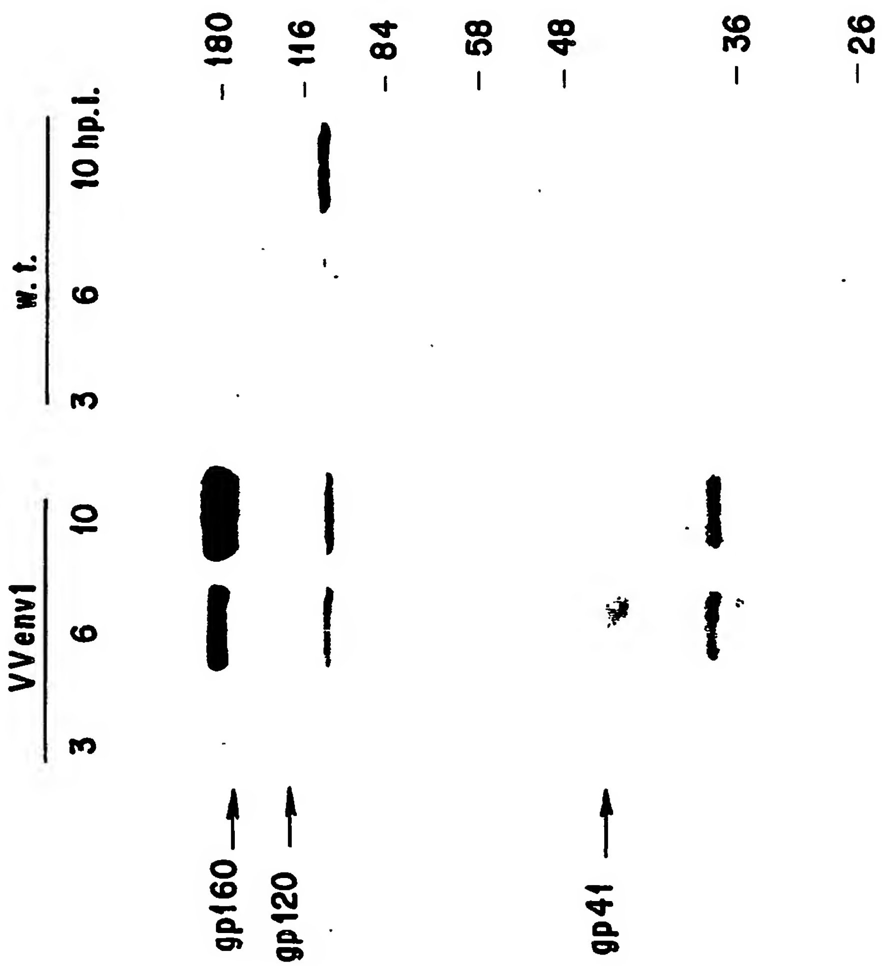

VVenv1
w.t.
3
6
10
3
6
10 hp.i.
gp160
gp120
gp41
180
116
84
58
48
36
26

FIG. 3a

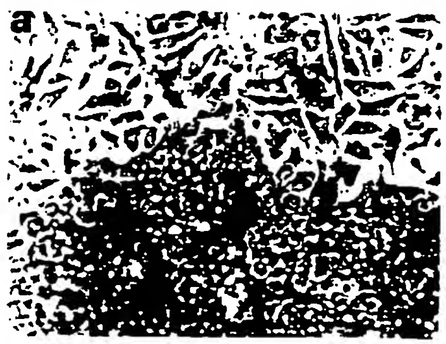


FIG. 3b

FIG. 3c

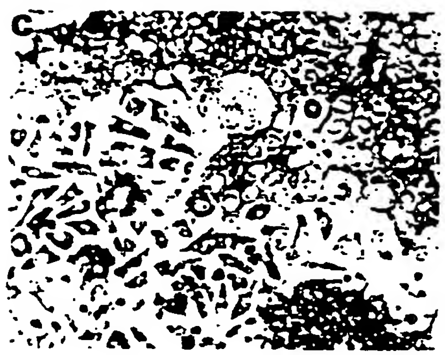


FIG. 3d

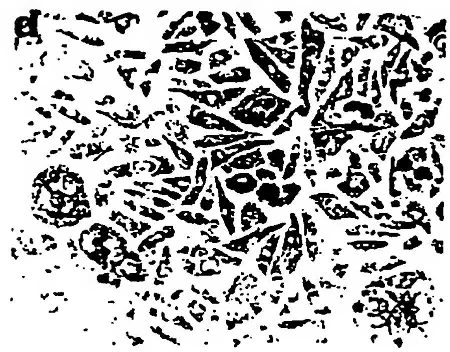
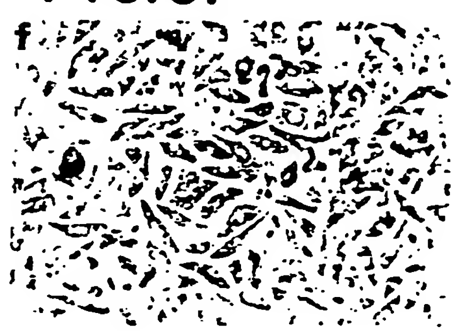


FIG. 3e

FIG. 3f



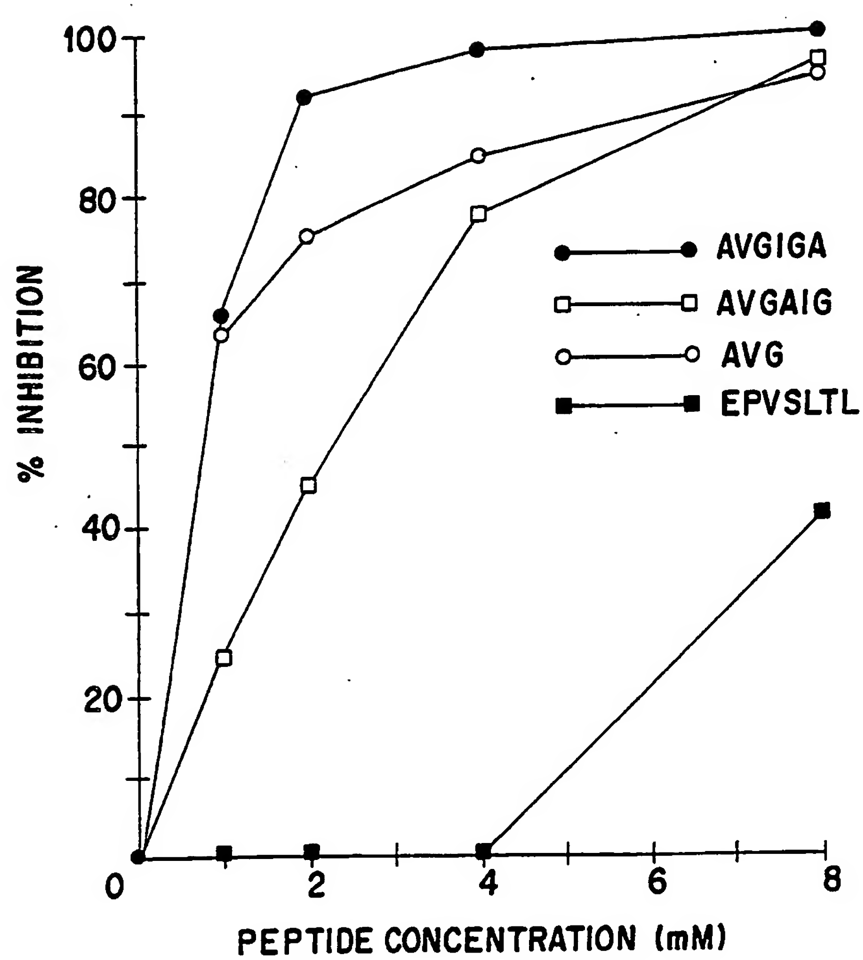
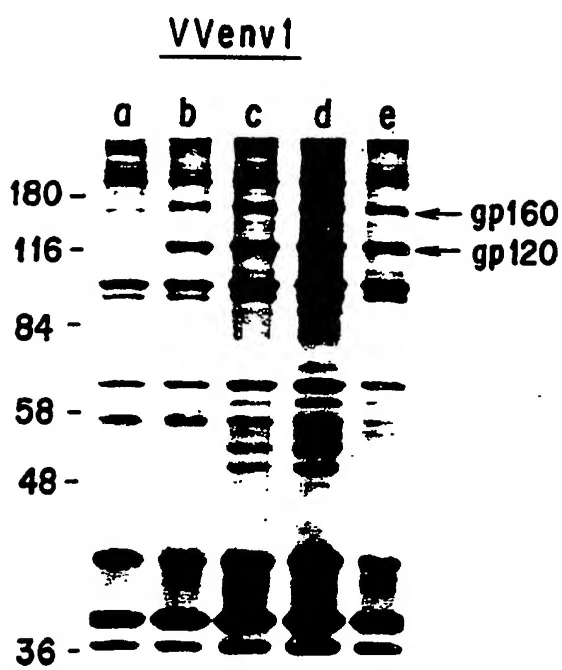

100
80
60
40
20
0
2
4
6
8
% INHIBITION
PEPTIDE CONCENTRATION (mM)
AVGIGA
AVGAIG
AVG
EPVSLTL

FIG. 4

FIG. 5



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 89/01426

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl(4) - C07K 7/10
U.S. Cl. - 530/350,324,327

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/350, 324, 327

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A, P	US,A, 4,812,556, (VAHLNE), 14 March 1989 See the entire article.	1-45
A, P	US,A, 4,772,547 (HEIMER), 20 September 1988.	1-45
A	Chemical Abstracts, Volume 105, No. 103, issued 1988 (Columbus, Ohio, USA), Dalgleish, "Neutralization of diverse HIV-1 strains by monoclonal antibodies raised against a gp41 synthetic peptide", see the entire article, column 1, abstract No. 108673e, Virology 1988, 165(1), 209-15 (Eng).	1-14, and 23-44
A	Chemical Abstracts, Volume 108, issued 1988, (Columbus, Ohio, USA), Hellewell, "A monocyte Chemotaxis inhibiting factor in serum of HIV infected men shares epitopes with the HIV transmembrane protein gp41", see the entire article, col. 2, abstracts no. 92905y, clin. Exp. Immunol. 1988, 71(1), 13-18(Eng).	1-14 and 23-44

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 July 1989	13 SEP 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Teresa D. Wessendorf Teresa D. Wessendorf

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Proc. Natl. Acad. Sci. USA, Vol. 83, December 1986, Reiher III, "Sequence homology between acquired immunodeficiency syndrome virus envelope protein and interleukin 2", pages 9188-9192, see page 9191, columns 1 and 2.	1,8,29, 36,43 and 44
A	Cell, Volume 45, 06 June 1986, Starchich, "Identification and characterization of Conserved and Variable Regions in the envelope gene of HTLV-111/Lav, the retrovirus of Aids", pages 637-648 , See the entire document.	1,8,9, 43 and 44
X	Gene, vol. 64, 1988, Windheuser, "Characterization of immunoreactive epitopes of the HIV-1 p 41 envelope protein using fusion proteins snythesized in Escherichia coli", pages 107-119 See the entire document.	1,8 ,15 22, 29, 36 and 43-45
A	Nature, Volume 326, 09 April 1987, Kornfeld, "Cloning of HTLV-4 and its relation to simian and human immunodeficiency viruses", pages 610-613, see the entire document.	15,22,43 and 45
Y	Nature, Volume 326, 16 April 1987, Guyader, "Genome organization and transactivation of the human immunodeficiency virus type 2", pages 662-669, see the entire document.	15,22,43 and 45
Y	Nature, Volume 324, 18/25 December 1986, Clavel, "Molecular cloning and polymorphisn of the human immune deficiency virus type 2", pages 691-695, See the entire document.	15,22, 43 and 45
X	The Journal of Biological Chemistry, Vol. 262, No. 12, 25 April 1987, Kennedy, "use of a Resin-bound Synthetic peptide for identifying a neutralizing antigenic determinant associated with the human immunodeficiency virus envelope", pages 5769-5774, See the entire document.	1,8,29, 36,43 and 44
X	FEBS LETTERS, Volume 218, No. 2, June 1987, Sternberg, "Prediction of Antigenic determinants and secondary structures of the major AIDS Virus proteins", pages 231-237, see the entire document	1,8,29, 36,43 and 44

International Application No. PCT/US 89/01426

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter[12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out[13], specifically:

3. ☐ Claim numbers__________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. X OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

See attachment.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. X As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

The elected last specie of each claims 1, 8, 15, 22, 29 and 36.

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

PCT/US 89/01426

Attachment to form PCT/ISA/210, VI.

Group I - Claim 1
Species 1-30 -dipeptide to peptides containing more or less thirty amino acid residues.

Group II - Claim 8
Species 1-31 - dipeptide to peptides containing more or less thirty-one amino acid residues.

Group III - Claim 15
Species 1-36 - dipeptide to peptides containing more or less thirty-six amino acid residues.

Group IV - Claim 22
Species 1-36 - dipeptide to peptides containing more or less thirty-six amino acid residues.

Group V - Claim 29
Species 1-13 - dipeptide to peptides containing more or less thirteen amino acid residues.

Group VI - Claim 36
Species 1-13 -dipeptide to peptides containing more or less thirteen amino acid residues.

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☑ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: ______________________

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.